SOME CHARACTERISTICS OF α_s- AND β-CASEIN VARIANTS

In 1962, we reported the discovery of genetic variants of α_s-casein of cow's milk (7). Three electrophoretic forms of α_s-casein can occur singly (A, B, or C) or in pairs (AB, AC, or BC) in the milk of individual cows, an observation which parallels the studies of Aschaffenburg (1) on β-caseins. The genetics of α_s-casein polymorphism is described by Kiddy et al. (5).

This report describes some of the characteristics of purified α_s- and β-caseins. Additional properties and a more detailed report will appear in a subsequent publication. Each of the genetic variants of α_s- and β-caseins was prepared from the milks typed homozygous for the particular variant. All but β-casein C were available, and whole casein of this type was a gift of Dr. R. Aschaffenburg, Reading, England.

Briefly, the preparation of α_s-casein involved the isolation of whole α-casein by the method of Hipp et al. (3), followed by the separation of crude α_s-casein with calcium ions at 0-4 C. The α_s-casein was further purified by precipitation of contaminants with the addition of ammonium acetate to a 50% ethanol-water solution of the α_s-casein (11). Final purification was accomplished by DEAE-cellulose chromatography in the presence of 3.3 M urea. β-Casein, prepared by the method of Hipp et al. (3), was also purified by column chromatography.

By starch-gel-electrophoresis (9) and polyacrylamide-gel-electrophoresis, each of the α_s- and β-caseins was essentially free of contamination. The α_s-caseins formed stable micelles with κ-casein in the presence of calcium ions, and in the ultracentrifuge appeared free of contamination. However, the aggregation behavior of the three variants differed. The association behavior of the β-casein variants, and the formation of β-κ complexes as revealed by ultracentrifugation, are being studied.

Table 1 shows the phosphorus and nitrogen contents of α_s- and β-casein variants, and the corresponding P/N ratio. The α_s-casein variants, like $\alpha_{s1,2}$ (10) (probably α_s-BC), have the same phosphorus content. However, the individual variants show higher nitrogen values than either $\alpha_{s1,2}$ or α_1-casein (6). As can be observed, the P/N ratios are different because of the differences in the nitrogen contents of the variants. Of interest is the observation that all α_s-caseins contain nine residues of phosphorus, assuming a molecular weight of 27,500 (10) for each variant.

TABLE 1

Nitrogen and phosphorus analyses[a] of α_s-A, B, and C and β-A, B, and C caseins

Variant	Phosphorus	Nitrogen	P/N ratio	Atoms P/mol
	(%)	(%)		
α_s-A	1.01	15.10	0.0668	9.0[b]
α_s-B	1.01	15.34	0.0658	9.0
α_s-C	1.01	15.40	0.0655	9.0
β-A	0.59	15.18	0.0389	4.8[c]
β-B	0.57	15.33	0.0372	4.6
β-C	0.50	15.45	0.0324	4.0

[a] Moisture- and ash-free basis.
[b] Assuming a molecular weight of 27,500.
[c] Assuming a molecular weight of 25,000.

β-Casein contains considerably less phosphorus than α_s-casein, and the values correspond closely to those reported by Hipp et al. (3) for β-casein prepared from pooled milk. The nitrogen values, although consistent with those of Hipp et al., differ among the variants. Assuming a molecular weight of 25,000, β-A, B, and C contain 4.8, 4.6, and 4.0 residues of phosphorus, respectively. Considering the reliability of the phosphorus analyses ($\pm$0.03%), the number of residues of phosphorus for β-A and B is probably five. However, the molecular weights among the variants may differ, giving rise to an apparent nonintegral number of phosphorus atoms.

The difference in electrophoretic mobilities of α_s- and β-casein variants cannot be explained on the basis of their phosphorus contents. Kalan et al. (4) reported that α_s-A, B, and C all possess the same end groups, C-terminal tryptophan, and N-terminal arginine. Similar information is as yet unavailable for the β-caseins. By peptide mapping (4) and by amino acid analyses (2, 8), it is certain that amino acid addition/deletion or substitution has occurred within the polypeptide chains of the genetic variants of α_s-caseins. Similar (unpublished) evidence suggests the same to be true of β-caseins. Our efforts are continuing in the direction of attempting to determine the exact chemical differences among the genetic variants of these proteins.

M. P. THOMPSON
AND
L. PEPPER
Eastern Regional Research Laboratory[1]
Philadelphia, Pennsylvania

[1] Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

REFERENCES

(1) ASCHAFFENBURG, R. Inherited Casein Variants in Cow's Milk. Nature, 192: 431. 1961.
(2) GORDON, W. G., AND BASCH, J. J. Differences in Amino Acid Composition of Genetic Variants of Bovine α_s-Caseins. Federation Proc., 22: 657. 1963.

(3) HIPP, N. J., GROVES, M. L., CUSTER, J. H., AND MCMEEKIN, T. L. Separation of α, β, and γ-Casein. J. Dairy Sci., 35: 272. 1952.
(4) KALAN, E. B., GREENBERG, RAE, THOMPSON, M. P., AND PEPPER, L. Some Chemical Characteristics of α_s-Caseins. Federation Proc., 22: 657. 1963.
(5) KIDDY, C. A., JOHNSTON, J. O., AND THOMPSON, M. P. Genetic Polymorphism in Caseins of Cow's Milk. I. Genetic Control of α_s-Casein Variation. J. Dairy Sci., 47: 147. 1964.
(6) MCMEEKIN, T. L., HIPP, N. J., AND GROVES, M. L. The Separation of the Components of α-Casein. I. The Preparation of α_1-Casein. Arch. Biochem. Biophys., 83: 35. 1959.
(7) THOMPSON, M. P., KIDDY, C. A., PEPPER, L., AND ZITTLE, C. A. Variations in the α_s-Casein Fraction of Individual Cow's Milk. Nature, 195: 1001. 1962.
(8) THOMPSON, M. P., PEPPER, L., GORDON, W. G., AND BASCH, J. J. Chemical Composition of Bovine α_s, A, B, and C Caseins. J. Dairy Sci., 46: 607. 1963.
(9) WAKE, R. G., AND BALDWIN, R. L. Analysis of Casein Fractions by Zone Electrophoresis in Concentrated Urea. Biochim. et Biophys. Acta, 47: 225. 1961.
(10) WAUGH, D. F., LUDWIG, MARTHA L., GILLESPIE, J. M., MELTON, BETTYE, FOLEY, MARGARET, AND KLEINER, ELIZABETH S. The α_s-Caseins of Bovine Milk. J. Am. Chem. Soc., 84: 4929. 1962.
(11) ZITTLE, C. A., AND CUSTER, J. H. Purification and Some Properties of α_s-Casein and κ-Casein. J. Dairy Sci., 46: 1183. 1963.